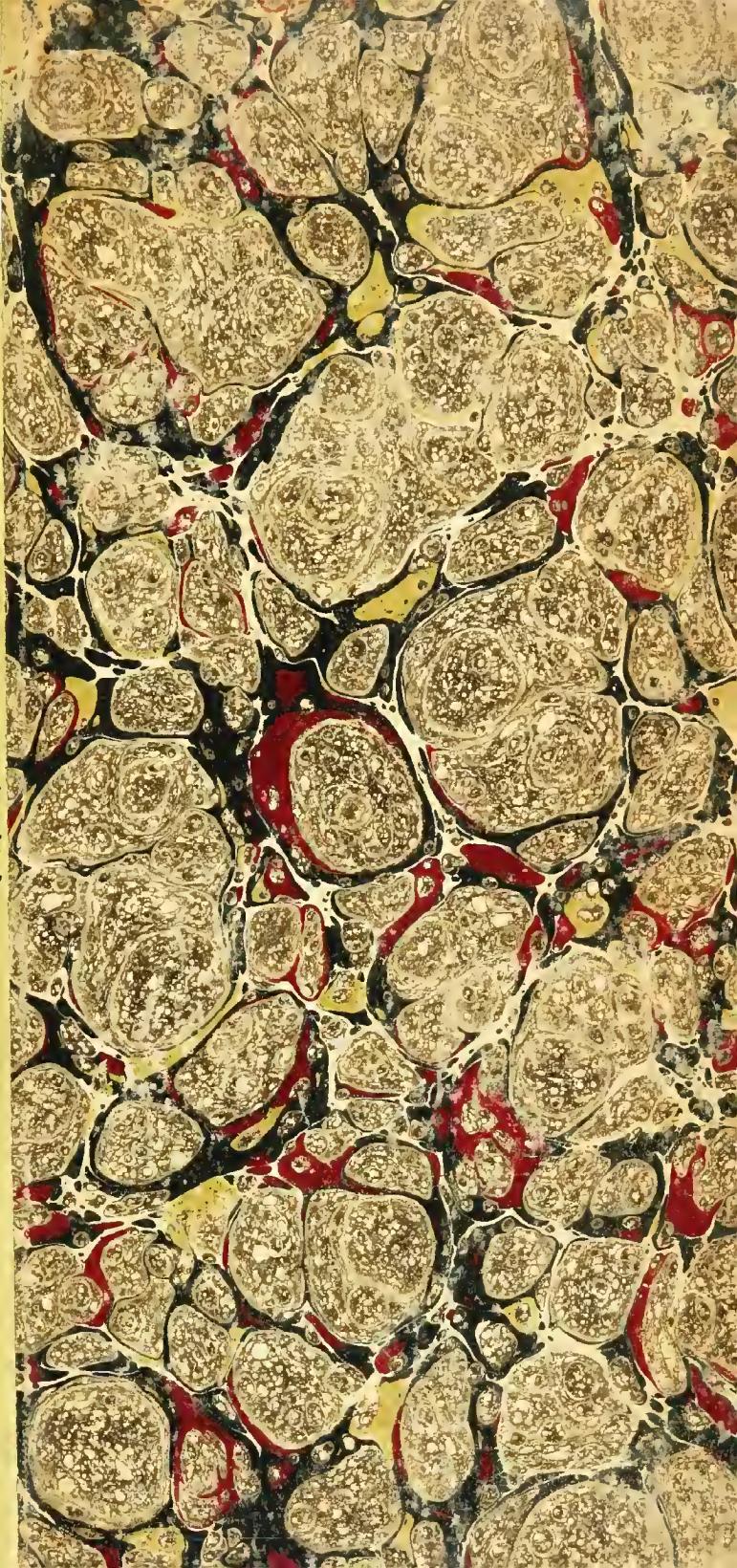


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1897

EXERCISES
IN
PRACTICAL PHYSIOLOGY

BY
AUGUSTUS D. WALLER, M.D., F.R.S.

PART I.
ELEMENTARY PHYSIOLOGICAL
CHEMISTRY

BY
AUGUSTUS D. WALLER
AND
W. LEGGE SYMES.

LONGMANS, GREEN, AND Co.
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ELEMENTARY PHYSIOLOGICAL CHEMISTRY.



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GENERAL DIRECTIONS.

No 840

Ascertain the reaction of every fluid that you test, by dipping strips of litmus paper in it. Acid fluids make neutral, or blue litmus paper red. Alkaline fluids make neutral, or red litmus paper blue.

To neutralise a fluid,—add a .1 per cent. solution of caustic soda, or a .1 per cent. solution of hydrochloric acid, until it does not change the colour of neutral litmus paper.

The specific gravity of a fluid is measured by immersing a hydrometer in it, and noting to what degree the instrument sinks.

Unless otherwise directed, you are to use fresh material for each reaction.

EXAMINATION OF THE MORE IMPORTANT PROXIMATE PRINCIPLES.

PROTEIDS.

GENERAL REACTIONS.

<i>Directions.</i>	<i>Result.</i>
1 Burn a little dry proteid with soda-lime in a spoon <i>The charring proves the presence of carbon, the smell shows that nitrogen is present.</i>	It becomes charred, and smells of ammonia.
2 <i>Xanthoproteic reaction.</i> Add nitric acid to a proteid solution and boil then cool, and add ammonia	A yellow colouration or precipitate appears. The colour deepens to orange.
3 <i>Biuret reaction.</i> ¹ Add one drop of copper sulphate solution, and one volume ² of strong caustic soda solution	A violet or pink colour results.
4 Add $\frac{1}{4}$ volume of Millon's re-agent .. then boil the solution	A precipitate is formed. The precipitate becomes reddish.
5 Add $\frac{1}{4}$ volume of potassium ferrocyanide solution, and excess (one volume or more) of acetic acid	A precipitate appears.
<i>Peptones and some albumoses do not give this reaction.</i>	
6 Saturate ³ with sulphate of ammonium ..	A precipitate forms.
<i>Peptones are not precipitated.</i>	

¹ Properly speaking, the term "biuret reaction" is applicable only to the pink colour obtained with digested proteids, and with biuret itself.

² "One volume" means an equal bulk.

³ You will not really saturate the fluid. To do so would require prolonged agitation. It will be enough for you to shake the solution for five minutes with half its bulk of crystals. You may see the gradual formation of precipitate by pouring fluid on to a layer of crystals and leaving it undisturbed in a test tube. As the salt dissolves a turbid ring forms at the junction of fluid and crystals. This applies also to the globulin test by magnesium sulphate.

SPECIAL REACTIONS.

*Directions.**Result.***Native albumins** (*solutions of egg albumin, and of serum albumin free from globulins*).

- | | |
|---|--|
| 7 Faintly acidify and boil | The albumin is coagulated. |
| 8 Saturate with sulphate of magnesium .. | The albumin is <i>not</i> precipitated. |
| 9 Acidify, add one volume of ether, and shake | <div style="display: inline-block; vertical-align: middle;"> <div style="font-size: 3em; vertical-align: middle; margin-right: 5px;">{</div> <div> Egg albumin is usually coagulated.
 Serum albumin is <i>not</i> usually coagulated. </div> </div> |

Globulins (*solution of serum globulin, or of muscle globulin*).

- | | |
|---|---|
| 10 Faintly acidify, and boil | The globulin is coagulated. |
| 11 Saturate with sulphate of magnesium .. | The globulin is precipitated. |
| 12 Let a few drops fall into distilled water .. | A cloud, <i>i.e.</i> , a faint precipitate of globulin appears. |

Derived albumins (*prepared by treating native albumins, or globulins with dilute acid or alkali*).

- | | |
|---|---|
| 13 Take reaction | It is acid, or alkaline, <i>not</i> neutral. |
| 14 Boil | Derived albumins are not coagulated. |
| 15 Cautiously neutralise, shaking after each addition of neutralising re-agent, then add slight excess of the neutralising re-agent | Derived albumins are precipitated.
The precipitate re-dissolves. |

Albumoses—

- | | |
|---|--|
| 16 Faintly acidify, and boil | Albumoses are not coagulated. |
| 17 Apply biuret test | A pink colour results. |
| 18 Saturate with sulphate of magnesium .. | Some albumoses (primary albumoses) are precipitated. |
| 19 Cautiously add nitric acid, or salicyl-sulphonic acid, and shake | A precipitate forms. |
| then heat the test tube | The precipitate dissolves. |
| and then cool the test tube under the tap | The precipitate re-appears. |

Peptones—

- | | |
|--------------------------------------|--------------------------------|
| 20 Faintly acidify, and boil | Peptones are not coagulated. |
| 21 Apply the biuret test | A pink colour results. |
| 22 Add nitric acid | Peptones are not precipitated. |

COMPOUND PROTEIDS.

*Directions.**Result.***Mucin** (*a compound of proteid and sugar*).

- | | |
|-------------------------------------|--|
| 23 Boil | Mucin is not coagulated. |
| 24 Apply xanthoproteic test | Yellow colour results. |
| 25 Add acetic acid | Mucin is precipitated, and does not re-dissolve in excess of the acid. |

Nucleo-albumins (*compounds of proteid with nuclein*).

- | | |
|---|-----------------------------------|
| 26 Apply the xanthoproteic test | An orange colour results. |
| 27 Add acetic acid | Nucleo-albumins are precipitated. |
| 28 Saturate with magnesium sulphate | Nucleo-albumins are precipitated. |

Nucleo-albumins react very much like globulins, and like mucin.

Oxyhæmoglobin (*a compound of proteid with hæmatin, and oxygen*).

- | | |
|--|---|
| 29 Boil | The proteid is coagulated, and the hæmatin (brownish pigment) liberated. |
| 30 Dilute and examine with a spectroscope | Two dark absorption bands are seen between the yellow and green of the spectrum. |
| 31 Add sulphide of ammonia to remove the oxygen, and then examine as in 30 | The two bands vanish, and a single, broader, fainter band appears in their place. The oxyhæmoglobin is reduced to hæmoglobin. |
| 32 Add tincture of guaiacum, and ozonic ether | A blue colour is developed. |

ALBUMINOID.

Gelatin—

- | | |
|---|------------------------------|
| 33 Warm the jelly | It becomes fluid. |
| then cool the fluid under the tap | It gelatinises. |
| 34 Apply the biuret test | A violet colour results. |
| 35 Add nitric acid | Gelatin is not precipitated. |
| then complete the xanthoproteic reaction | A yellow colour results. |
| 36 Apply reaction No. 5 | Gelatin is not precipitated. |

If you do not dilute sufficiently, you may see a single very distinct broad absorption band. You must not confuse this with the single faint band of reduced hæmoglobin.

SUMMARY OF THE CHIEF REACTIONS OF THE PROTEIDS.

Coagulate on boiling	{ Egg albumin	Coagulated by ether, not precipitated by sulphate of magnesium.
	{ Serum albumin....	Not coagulated by ether, not precipitated by sulphate of magnesium.
	{ Globulins	Precipitated by saturation with sulphate of magnesium.
Do not coagulate on boiling	{ Derived albumins..	<i>Violet</i> biuret reaction. Precipitated on neutralisation.
	{ Albumoses	<i>Pink</i> biuret reaction. Precipitated by nitric acid and by salicyl-sulphonic acid, the precipitate disappearing with heat, and re-appearing on cooling the solution. Precipitated on saturation with sulphate of ammonium. ¹
	{ Peptones.....	Pink biuret re-action in filtrate after saturation with sulphate of ammonium.

FAT.

*Directions.**Result.*

- | | |
|--|--|
| 37 Shake up some rancid oil (<i>i.e.</i> , liquid fat <i>plus</i> fatty acid) with water then add some alkali and shake again | The fat is neither dissolved nor emulsified.
The fat is not dissolved, but is emulsified. |
| 38 Shake up one volume of oil with four or five volumes of ether then pour a little of the ethereal solution on a piece of paper | The fat dissolves.

The ether evaporates leaving a permanent greasy stain. |
| 39 Take one or two drops of emulsified oil, and boil with alkali | The fat dissolves, yielding glycerine, and forming soap. |

¹ Subject to the qualifications adverted to in Part II.

CARBO-HYDRATES.

	<i>Directions.</i>	<i>Result.</i>
Starch—		
40	Acidify and add iodine solution	A blue colour results.
	then heat the blue fluid	The colour disappears.
	and then cool the colourless solution ..	The colour comes back.
Glycogen—		
41	Acidify and add iodine solution	Reddish brown colour.
	then heat the brown fluid	The colour vanishes.
	and then cool the colourless solution ..	The colour <i>often</i> reappears.
42	Add two or three volumes of alcohol ..	Glycogen is precipitated.
Dextrin—		
43	Acidify and add iodine solution	Reddish brown colour.
	then heat the brown fluid	The colour vanishes.
	and then cool the colourless solution ..	The colour <i>may</i> reappear.
Dextrose—		
44	<i>Trommer's test.</i>	
	To some caustic soda in a test tube, add one or two drops of copper sulphate ..	A bluish precipitate (cupric hydrate) forms.
	then add a little solution of dextrose ..	The precipitate dissolves, giving a blue solution.
	and then boil	The cupric hydrate is reduced giving a red precipitate of cuprous oxide.
45	<i>Moore's test.</i>	
	Add one volume of caustic soda solution and boil	A yellow, or brownish colour (caramel) results, according to the amount of sugar present.
<i>Moore's test is only to be used as a rough test for dextrose in the presence of proteids.</i>		
Lactose—		
46	Apply Trommer's test	The cupric hydrate is reduced as by dextrose.
Saccharose—		
47	Apply Trommer's test	The cupric hydrate is <i>not</i> reduced, and so no red precipitate is formed.
48	Boil with a few drops of dilute hydrochloric acid, to convert the saccharose into reducing sugar, and apply Trommer's test.	A red precipitate results, as with dextrose.

EXAMINATION OF THE MORE IMPORTANT ARTICLES OF DIET.

BREAD.

<i>Directions.</i>	<i>Result.</i>	<i>Inference.</i>
49 Pour a drop of iodine solution on bread	A blue colour is developed	Starch is present.
50 Apply the xanthoproteic reaction to another piece	An orange colour results	Proteid is present.

POTATO.

51 Boil a bit of potato in water, cool, and add some iodine solution	A blue colour appears	Starch is present.
<i>You could not detect the starch without boiling because the granules are enclosed in a coating of cellulose.</i>		
52 Apply the xanthoproteic test	Only a faint orange colour appears	Very little proteid is present.

BUTTER.

53 Shake up a little butter with some ether.	It dissolves, leaving little or no residue	It is composed almost entirely of fat.
--	--	--

COMMERCIAL "PEPTONE."

54 To a little solution of commercial peptone, apply the biuret test	Pink colour results	The only proteids that can be present are peptones and albumoses.
55 Saturate some of the solution with ammoniac sulphate.	A copious precipitate falls	Much albumose is present.
56 Remove the precipitated albumose by filtration and apply the biuret test to the filtrate. ¹	A faint pink colour may develop	If so a little peptone is present.

¹ Great excess (4 or 5 volumes or more) of caustic soda is necessary.

BEEF TEA (HOT) MADE BY BOILING MEAT AND BONES.

<i>Directions.</i>	<i>Result.</i>	<i>Inference.</i>
57 Cool some under the tap	It gelatinises ..	Gelatin is present.
58 Apply the xantho-proteid test	A faint yellow colour results, <i>not</i> usually <i>orange</i>	Little, if any, proteid is present.
59 Shake a little with ether and pour the ether on some paper.	A slight greasy stain may remain	If so a little fat is present.
60 Add a little sodium nitro-prusside and caustic soda then boil and while boiling add acetic acid	A red colour appears The colour fades A blue or green colour results	 Creatinin is present.

BEEF TEA MADE WITH EXTRACT OF MEAT¹ ('LIEBIG').

61 Apply the xanthoproteic test	A faint yellow colour appears	Little, if any, proteid is present.
62 Shake a little with ether, and pour the ether on a filter paper	No greasy stain results	Fat is absent.
63 Add some iodine solution	A faint brown colour may appear	If so, a little glycogen or dextrin ² is present.
64 To another portion add sodic nitro-prusside and caustic soda then boil and while boiling add acetic acid	A red colour develops The colour fades A blue or green colour appears	 Creatinin is present.

¹ This fluid has been decolourised by shaking it with animal charcoal and filtering.

² The body is present in such small quantity that you cannot tell whether it is glycogen or dextrin. You would expect to find the former rather than the latter.

EXAMINATION OF THE MORE IMPORTANT TISSUES AND FLUIDS OF THE BODY.

SALINE EXTRACT OF LIVER, *i.e.*, OF EPITHELIAL CELLS.

<i>Directions.</i>	<i>Result.</i>	<i>Inference.</i>
65 Apply the xanthoproteic test	Deep orange colour	Proteid is present.
66 Faintly acidify, and boil	Coagulation occurs	This proteid is either native albumin, or globulin, or both.
67 Saturate with sulphate of magnesium	Precipitation ¹ occurs	Some of the proteid may be globulin.
68 Filter the mixture obtained in test 67 and boil	Little or no coagulation occurs	Little or no albumin is present.

BONE ASH.

69 Dissolve bone ash in dilute nitric acid, warming, to accelerate solution	Effervescence occurs, the gas being colourless	Some carbonate is present.
70 To the solution add ammonic molybdate, and boil	A copious crystalline yellow precipitate falls	Much phosphate is present.

SALINE EXTRACT OF NERVOUS TISSUE.

71 To a saline extract of brain, apply the xanthoproteic test	Orange colour results	Proteid is present.
72 Faintly acidify another portion, and boil <i>The solids of a saline extract of brain are derived chiefly from the grey matter.</i>	The proteid is coagulated	The proteid is native albumin or globulin or both.

ETHEREAL EXTRACT OF NERVOUS TISSUE.

73 Pour a little of an ethereal extract of brain on a filter paper	A permanent greasy stain results	Fat is present. ²
74 To another portion add cautiously some strong sulphuric acid	A cherry red colour results at the junction of the two fluids	Cholesterin is present.

The solids of an ethereal extract of brain are derived chiefly from the white matter.

¹ The precipitation is not entirely due to globulin, but in part to nucleo-albumin.

² Remember that this is not wholly ordinary fat, but largely a nitrogenous, phosphorised fat,—lecithin.

Mar. 10

BLOOD (DEFIBRINATED).

<i>Directions.</i>	<i>Result.</i>	<i>Inference.</i>
Observe the specific gravity of defibrinated blood	It is between 1050 and 1060.	
75 Dip a piece of neutral glazed litmus paper in the blood, and wash away the blood-stain.	The paper is turned blue	Blood is alkaline.
76 Dilute freely, add a little freshly made tincture of guaiacum, and ozonic ether	A blue colour is formed.	Hæmoglobin ¹ is present.
77 Dilute freely ² and examine with the spectroscope	Two dark absorption bands are seen between the yellow and green of the spectrum	Oxy-hæmoglobin was present and has been deoxygenated.
78 Add sulphide of ammonium (a reducing agent) and warm gently	The two bands vanish and a single, broader, fainter band appears in their place	
79 Pour the fluid backwards and forwards in two test tubes	The two bands temporarily re-appear	The hæmoglobin has been temporarily re-oxygenated.
80 Dilute and examine with the spectroscope, blood saturated with coal gas	Two bands like those seen in No. 77 appear	Carboxy-hæmoglobin is present.
81 Add sulphide of ammonium, and warm gently	The spectrum is not altered	
82 Dry a little blood on a slide, add glacial acetic acid, cover, heat until bubbles appear, and examine with microscope	Nut-brown crystals of hæmin are seen	Hæmoglobin is present.

¹ This is not a distinctive test for hæmoglobin.

² If you do not dilute sufficiently, you may see a single very distinct broad absorption band. You must not confuse this with the single faint band of reduced hæmoglobin.

BLOOD ASH.

<i>Directions.</i>	<i>Result.</i>	<i>Inference.</i>
83 Place blood-ash in a test tube, dissolve it in hydro-chloric acid, and add ferrocyanide of potassium	A blue colour forms	Iron is present in blood.

BLOOD SERUM (DILUTED).

84 Apply the xanthoproteic test to some of the fluid	Orange colour results	Proteid is present.
85 Boil another portion . .	Coagulation takes place	The proteid is native albumin, or globulin, or both.
86 Saturate with sulphate of magnesium	A precipitate falls	Globulin is present ("serum globulin").
87 Remove the precipitated globulin by filtration, acidify the filtrate and boil	Coagulation occurs	Native albumin is present ("serum albumin").

SALINE EXTRACT OF MUSCLE.

88 Apply the xanthoproteic test to one portion	An orange colour results	Proteid is present.
89 Boil another portion . .	Coagulation occurs	The proteid is native albumin, globulin, or both.
90 Saturate with sulphate of magnesium	A precipitate falls	Globulin is present (chiefly myosin).
91 Remove the precipitated globulin by filtration, acidify the filtrate and boil	Coagulation occurs	A native albumin is present (muscle-albumin).

MILK.

<i>Directions.</i>	<i>Result.</i>	<i>Inference.</i>
92 Observe specific gravity	It is between 1025 and 1035	
93 Dip neutral litmus paper in the milk	It should become blue	Normal milk is alkaline.
<i>London Milk is frequently acid.</i>		
94 Dilute a little milk 3 or 4 times, add a few drops of <i>dilute</i> acetic acid, and warm Filter off the precipitate and put it aside.	A precipitate ¹ falls.	
95 To some of the <i>filtrate</i> obtained in No. 94 apply Trommer's reaction	A red precipitate is formed	A reducing sugar is present (lactose).
96 To another portion of the filtrate obtained in No. 94 apply the xanthoproteic reaction	Orange colour results	Proteid is present (milk albumin).
97 Take the precipitate obtained in No. 94 and wash it with ether. Pour a little of the ethereal solution on a piece of paper	Permanent greasy stain	Fat is present.
98 Dissolve the residue left from No. 97, in dilute alkali and apply the xanthoproteic test	Orange colour results	Proteid is present (caseinogen).
99 To some warmed milk add a little rennet and set aside for 5 or 10 minutes	The milk clots ¹ and forms "curd and whey"	

¹ The precipitate obtained by acid and the clot obtained by rennet are different bodies. The acid precipitate is "caseinogen" and is freely soluble in dilute alkali; the rennet clot is "casein" and is much less soluble in dilute alkali. Cheese is made with rennet and cannot be made with acid. Caseinogen is a nucleo-albumin.

URINE.

<i>Directions.</i>	<i>Result.</i>	<i>Inference.</i>
Observe the specific gravity	It is between 1015 and 1025.	
100 Dip a neutral litmus paper into some normal urine	It is turned red..	Normal urine is acid.
101 Do the same with some stale urine	It is turned blue..	Stale urine is alkaliue.
102 Warm the paper used in No. 101	The blue fades ..	The alkalinity of stale urine is not due to fixed alkali, but to a volatile alkali (ammonium carbonate).
103 Add a few drops of silver nitrate solution and excess of nitric acid	A precipitate forms .	Chlorides are present.
104 Add a few drops of barium chloride solution and excess of hydrochloric acid	A precipitate forms	Sulphates are present.
105 Add nitric acid and boil, then add ammonium molybdate solution and boil again	A yellow precipitate forms	Phosphates are present.
106 Add solution of sodium hypobromite	Bubbles of gas (nitrogen) are evolved	A nitrogenous body is present.
107 Remove the sulphates and phosphates of urine by adding $\frac{1}{2}$ volume of baryta mixture, and filtering. To a portion of the filtrate add some mercuric nitrate solution	A precipitate forms (a compound of urea and mercury)	Urea is present.
108 To another portion of the filtrate obtained in No. 107, add some sodium chloride and then mercuric nitrate as before	No precipitate forms until excess of mercuric nitrate has been added	The presence of sodium chloride hinders the precipitation of urea.

	<i>Directions.</i>	<i>Result.</i>	<i>Inference.</i>
109	Add a little sodium nitro-prusside and caustic soda then boil and while boiling add acetic acid	A red colour develops. The colour fades. Colour changes to blue	Creatinin is present.
110	<i>Murexide test.</i> — Take some deposit obtained from a highly coloured, acid urine, and evaporate to dryness ¹ with a few drops of yellow nitric acid Then add a little dilute ammonia	As it dries it becomes reddish. The colour changes to violet	Uric acid is present.
111	<i>Schiff's test.</i> — Let a drop of an alkaline solution of uric acid fall on a filter paper, and then let a drop of silver nitrate solution fall on the paper near the other, so that they may touch	A dark line forms at the junction of the drops	Uric acid is a reducing agent.
112	Apply Trommer's test to the alkaline solution of uric acid, <i>not</i> to urine.	A red precipitate may be formed	Uric acid is a reducing agent. (This is a possible fallacy in testing urine for sugar.)

¹ Great care must be taken not to overheat the residue.

BILE.

<i>Directions.</i>	<i>Result.</i>	<i>Inference.</i>
Observe specific gravity	It is between 1020 and 1030	
113 Dip a neutral litmus paper in bile	It becomes blue..	Bile is alkaline.
114 Add acetic acid ..	A precipitate falls	Mucin ¹ is present.
115 <i>Pettenkofer's test</i> .— Shake up a little bile with a grain ² of cane sugar, and then add some strong sulphuric acid or a solution of furfural ² may be substituted for the sugar	The froth and fluid below it are coloured purple	Bile-salts are present.
116 <i>Gmelin's test</i> .—Smear bile evenly over a white tile and let a drop of yellow nitric acid fall into the film	Concentric rings of colour appear round the drop	Bile pigments are present.
117 To a solution of white gall stones in chloroform add some strong sulphuric acid	Cherry red colour appears	Cholesterin is present.

¹ This is not wholly mucin but in part nucleo-albumin.

² The purple colour tends to become black if too much sugar or furfural are used.

SALIVA.		
<i>Directions.</i>	<i>Result.</i>	<i>Inference.</i>
118 Take two test tubes A and B, and label them with your name. In A put starch solution and saliva, and in B starch solution and boiled saliva. Place both in the water bath for ten minutes. Meanwhile test saliva as follows: -		
119 Add excess of acetic acid	A precipitate falls	Mucin is present.
120 Apply the xanthoproteic test	An orange colour appears	Proteid is present.
121 Add silver nitrate and nitric acid	A precipitate falls	Chlorides ¹ are present.
122 Add ferric chloride ..	A red colour appears	A thiocyanate is present. ²
123 Divide the contents of test tube A into two parts, acidify one part and add iodine solution	A somewhat purple colour appears	Starch and dextrin are present (reddish brown, plus blue = purple).
	Or a reddish brown colour develops	In this case erythrodextrin and no starch is present.
	Or the solution remains colourless	In this case neither erythrodextrin nor starch is present.
124 To the other part apply Trommer's test	A red precipitate falls	Sugar is present (chiefly maltose).
125 Test some of the contents of tube B as in No. 123	A blue colour appears	} Starch is unaltered by boiled saliva
126 To the remainder apply Trommer's test	No red precipitate ³ falls	

¹ Silver nitrate precipitates both mucin and albumin, and so these bodies ought, properly speaking, to be removed before testing for chlorides, but the precipitate here is too copious to be entirely due to mucin and albumin.

² Assuming the absence of acetate and meconate.

³ A small precipitate may fall owing to presence of sugar in your saliva.

GASTRIC JUICE.

Vessels are placed on the tables labelled X and Y and containing products of gastric digestion.

<i>Directions.</i>	<i>Result.</i>	<i>Inference.</i>
127 Take two test tubes C and D and place a pellet of fibrin in each. To C add dilute gastric glycerin plus an equal volume of .3 per cent. hydrochloric acid, and to D add only .15 per cent. hydrochloric acid. Put them in the water bath and leave for 30 minutes Meanwhile examine the fluids X and Y. In X albumin has been digested with gastric juice, for a short time, in Y for a longer time.		
128 Neutralise a little of the fluid X	A precipitate falls on neutralisation	Acid albumin is present.
129 Apply the biuret test to a little of the fluid Y	A pink colour results	The only proteids present are albumose and peptone.
130 Neutralise, and saturate another portion with ammoniac sulphate	A precipitate falls	Albumose is present.
131 Remove the precipitated albumose by filtration and apply biuret test to the filtrate ¹	A pink colour results	Peptone is present.
132 Examine the tubes C and D. The fibrin in both is swollen.		
133 Filter their contents and apply biuret test to both filtrates	A pink colour results in C; a violet, in D	The fibrin in C is digested, that in D is <i>not</i> digested.

¹ In applying the biuret test to a filtrate after saturating with ammoniac sulphate it is necessary to add *great* excess (three or four volumes or more) of caustic soda. This dilutes the peptone considerably and so the pink will be much less distinct than in No. 129. For this reason you would probably fail to find peptone in the fluid X.

PANCREATIC JUICE.

Vessels labelled Z are placed on the tables containing products of the pancreatic digestion of proteids.

<i>Directions.</i>	<i>Result.</i>	<i>Inference.</i>
134 In a test tube E place a pellet of fibrin with a little dilute pancreatic glycerin, and an equal bulk of a 2 per cent. solution of sodic carbonate. Place in the water bath for 30 minutes.		
135 In another test tube F place some starch mucilage with pancreatic glycerin and sodic carbonate as above. Put it in the water bath for 10 minutes.		
136 In other test tubes G and H place some oil and add some blue litmus. To G add a small fragment of pancreas and place both in the water bath for 30 minutes. Whilst these "digests" are progressing, examine the fluid placed on the table; notice its smell.		
137 Neutralise some of it, and saturate it with sulphate of ammonium	Very little precipitate falls, if any	Digestion is practically complete.
138 Remove this precipitate, if necessary, by filtration and apply biuret test	A pink colour results	Peptone is present.

<i>Directions.</i>	<i>Result.</i>	<i>Inference.</i>
139 Dip the end of a wooden match into hydrochloric acid, and then into the fluid	The match may be stained red	If so indol is present.
140 Boil a little of the fluid with an equal volume of Millon's re-agent	A port wine colour may appear	If so, tyrosin is present
141 Examine the test tube E. The fibrin in it is eroded, not swollen as it was in No. 132		
142 Filter the contents of E and apply the biuret test to the filtrate	A pink colour results	The fibrin has been digested.
143 To the contents of F apply Moore's test	A yellow or brown colour results	The starch has been digested.
144 Compare the contents of G and H	In G the litmus has become red	Acid has been liberated by the pancreas.

ACTION OF INTESTINAL JUICE.

145 The vessel K contains saccharose. Apply Trommer's test to some of its contents	No red precipitate falls	The sugar present is not a reducing sugar.
146 The vessel L contained saccharose. But it has been acted on by succus entericus. To some of it apply Trommer's re-action	A red precipitate falls	The saccharose has been converted into a reducing sugar.

GENERAL HINTS FOR THE QUALITATIVE EXAMINATION OF SIMPLE FLUIDS CONTAINING SUBSTANCES OF PHYSIOLOGICAL INTEREST.

Take the reaction of the fluid. If it is coloured, examine it with the spectroscope, before and after the addition of ammoniac¹ sulphide.

Then decolourise with animal charcoal, and test the fluid for proteids. If proteids are present try and find out which proteids they are.

Test also for the albuminoids and ferments.

Whether proteids are present or not, test the original fluid for—

Starch	} acidifying if necessary.
Dextrin	
Glycogen	

Bile pigments

Uric acid	} using Schiff's test if the solution is alkaline, and the murexide in any case.
Digestive ferments (proteolytic alone, and with amylolytic ferments)	

Sulphates, phosphates, chlorides.

If proteids are absent test also for

Urea

Sugars

Bile salts

But if proteids are present take 25-50 cc. of the fluid, evaporate almost to dryness, extract residue with alcohol, filter, evaporate alcohol from filtrate, dissolve this residue in water, and then test for urea, sugars, and bile salts.

GENERAL HINTS FOR THE QUALITATIVE EXAMINATION OF SIMPLE SOLIDS CONTAINING SUBSTANCES OF PHYSIOLOGICAL INTEREST.

Examine microscopically, looking especially for—

Blood corpuscles,

Starch granules,

Crystals.

Make extracts with—

- | | |
|--|---------------------------------------|
| 1 Distilled water | } and examine them as directed above. |
| 2 Normal saline, and a 10 per cent. solution of sodic chloride | |
| 3 Alcohol | |
| 4 Ether, and look for fat. | |
| 5 Dilute acid, and look for phosphates. | |

If any insoluble residue remain it may be

Coagulated proteid,

Elastic tissue.

¹ Ammonic sulphide must not be added to an acid solution. If necessary therefore neutralise before reducing.

ESTIMATION OF SODIUM CHLORIDE IN URINE.

Mohr's Method—

You are given a burette containing a standard solution of silver nitrate, and a small flask. Place 10 cc. of urine in the flask with 100 cc. (half a flask), of distilled water, and 10 or 12 drops of potassium chromate solution. Let the silver solution run gradually into the urine, shaking the latter frequently until a *permanent* orange tinge appears in the precipitate.

Before calculating the amount of sodium chloride present, you must deduct 1 cc. from the amount of the standard solution used, because some of the silver is precipitated by the phosphates of the urine.¹ Then calculate as follows :—

Suppose that you required 10 cc. from the burette.

1 cc. of the standard solution precipitates .01 gram of sodic chloride.

∴ 9 cc. " " " .09 " " "
 ∴ .09 gram of sodic chloride are present in 10 cc. of urine.
 ∴ .9 " " " " 100 cc. " "

ESTIMATION OF THE PHOSPHORIC ACID IN URINE.

You are given a burette containing a standard solution of uranium acetate, a white basin, a white tile, and a vessel of potassium ferrocyanide solution.

Place a few drops of the ferrocyanide solution on different points of the tile. Place 50 cc. of urine in the basin, under the burette, and boil the urine; when it boils run in the standard solution gradually, stopping frequently, and testing some of the urine by dipping a glass rod in it, and then touching one of the ferrocyanide drops on the tile. When the ferrocyanide is tinged brown by the drop on the rod, the precipitation of the phosphoric acid is complete, and an excess of the uranium salt has been added. Suppose that you use 15 cc. from the burette, calculate as follows:—

1 cc. of standard solution = .005 gram of P_2O_5 .

∴ 15 cc. " " " = .005 × 15 gram of P_2O_5 .

∴ .005 × 15 grams of P_2O_5 are present in 50 cc. of urine.

Multiply this amount by 2 and you get the percentage of P_2O_5 in the urine. Remember that P_2O_5 does not exist *free* in the urine, but in combination with bases.

¹ In qualitative testing for the presence of chlorides, you prevent the formation of silver phosphate by adding nitric acid to the urine. You cannot add nitric acid here, because it would prevent the appearance of the orange colour, and you would not know when the precipitation was complete.

ESTIMATION OF DEXTROSE IN URINE.

Fehling's Method—

You are given a bottle of Fehling's solution, a white evaporating dish, and a burette containing a diabetic urine diluted ten times. Place the dish on a tripod under the burette, add 30 cc. of distilled water to it, and apply heat with a Buusen's burner. Note the level at which the solution in the burette stands, and when the Fehling boils run in the dilute urine, a few cc. at a time boiling after each addition, until the blue colour has nearly gone. Then work more cautiously, adding a few drops at a time until the Fehling is quite colourless. When all the blue is destroyed, note how much dilute urine you have used, and you will have a rough idea of the quantity required to decolourise 10 cc. of Fehling's solution. Now wash out the white dish and repeat the process adding, when the Fehling first boils, nearly enough dilute urine to decolourise it, and working gradually until you obtain exact decolourisation. To be accurate you ought to make a third determination, but for rough work two are enough.¹ Then find the average amount of solution used in your two or three determinations, and calculate the amount of sugar present in the urine as follows. Suppose that you used 12 cc. from the burette.

10 cc. of Fehling are decolourised by .05 gram dextrose

12 cc. of *diluted* urine contain .. .05 " "

.. 1 cc. " " " .. $\frac{.05}{12}$ " "

.. 100 cc. " " " .. $\frac{.05}{12} \times 100$ grams dextrose

.. 100 cc. *undiluted* " " .. $\frac{.05}{12} \times 100 \times 10$ grams dextrose

In estimating sugar observe the following cautions—

- 1.—Do not allow the Fehling to cool before looking to see whether it is colourless, because as it cools, it gets blue again.
- 2.—Do not trust a determination in which you require less than 10 cc. from the burette. If less than 10 cc. of the fluid decolourise your Fehling, the urine must be still further diluted, and of course this extra dilution must be allowed for in the calculation.
- 3.—Do not try to be *too accurate* in the determination of the end.

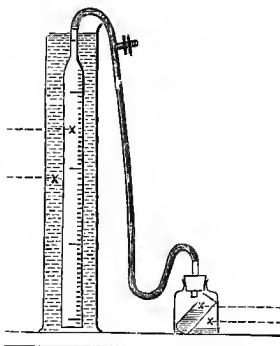
¹ If you have to do with a fluid, in which the percentage of sugar is *entirely* unknown to you, you may advantageously commence by estimating roughly and rapidly, applying the test to 1 cc. of Fehling diluted three times in a test-tube. If this is decolourised by less than 1 cc. of urine the amount of sugar is above 5 per 1,000, and the urine must be diluted. If, for example, you find with urine diluted ten times, that 2 cc. *do not*, and that 4 cc. *do* completely reduce 1 cc. of Fehling, you know that the sugar percentage in the diluted urine is between 2.5 and 1.25 per 1,000, and can then proceed to a more accurate determination within these limits.

ESTIMATION OF UREA IN URINE.

Hypobromite Method—

You are given a gas burette, standing in a tall jar of water, and connected by an india-rubber tube with a bottle, which has a small test tube in it, graduated to hold 5 cc.

Take out the graduated tube, and place 25 cc. of hypobromite of soda in the bottle. Pour urine into the graduated tube, up to the 5 cc. mark, and lower the tube carefully into the bottle containing the hypobromite solution. Then cork the bottle. This will force the level of the water inside the gas burette, below that outside it. Open the brass clip and the water in the burette will again rise to the proper level. Then close the clip and note the level of the water in the burette. Now tilt the bottle gently so as to mix the urine and hypobromite solution, and the urea will be decomposed into nitrogen, water, and carbonic acid gas. The carbonic acid will be absorbed by the free alkali in the hypobromite, and the nitrogen will pass over into the burette, and depress the water inside it. Allow the bottle to cool and then read the



amount of nitrogen evolved. This is done by lifting the burette until the water inside it is at the same level as the water outside it, and then noting the level of the water. Then, knowing the level at which the water stood before the experiment, you can ascertain the quantity of nitrogen evolved, and calculate the amount of urea present, as follows. Suppose that you obtain 30 cc. of nitrogen,

$$\begin{array}{llll}
 35.5 \text{ cc. of nitrogen is yielded by} & \dots & \dots & .1 \text{ gram of urea} \\
 \therefore 1 \text{ cc. of nitrogen} & \dots & \dots & \frac{.1}{35.5} \text{ } \\
 \therefore 30 \text{ cc. of nitrogen} & \dots & \dots & \frac{.1}{35.5} \times 30 \text{ } \\
 \therefore \frac{.1}{35.5} \times 30 \text{ gram of urea is present in 5 cc. of urine.} \\
 \therefore \frac{.1}{35.5} \times 30 \times 20 \text{ grams of urea are present in 100 cc. of the urine,} \\
 \text{\hspace{1.5cm} i.e., the percentage of urea in the urine is 1.69.}
 \end{array}$$

